

New Tools for Optogenetic Dissection of Pain Circuitry



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Disclosures



NeuroLux

Co-founder and part owner.

NeuroLux manufactures ultraminiaturized micro-LED devices for wireless optogenetics.

What is pain?

“An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” (IASP)

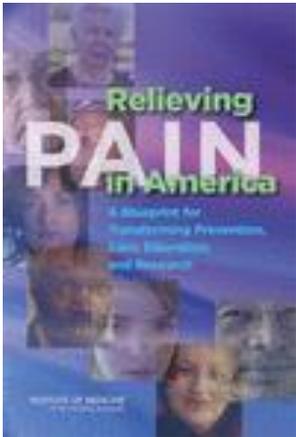


What is pain?



The Problem(s) of Pain

- **100 million** Americans suffer from chronic pain
- **\$560-635 billion:** Annual financial impact of chronic pain:
- **\$99 billion:** Direct cost in '08 to federal and state governments:



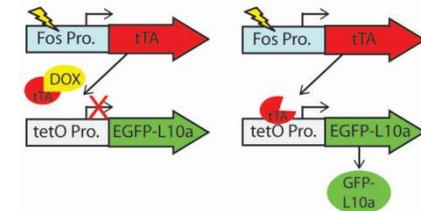
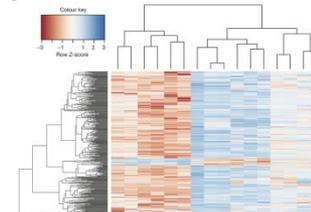
Source: Institute of Medicine, 2011

Technological Innovation Drives Advances in Our Understanding of Pain

- Molecular basis of pain transduction
 - TRP channels, ASICs, Piezos
- Functional and structural changes in the CNS associated with chronic pain
- Identification of genes that mediate congenital insensitivity/hypersensitivity to pain
- Understanding that chronic pain is a disease that needs to be understood and new treatments developed

New Technological Advances Driving Innovation in Neuroscience

- Deep sequencing
- “TRAP” approaches to gain genetic access to activated populations of neurons
- Enhanced anatomical techniques for trans-synaptic tracing
- In vivo imaging of circuit activity
- Techniques for somatic cell reprogramming to understand and treat disease
- Technologies enabling manipulation of identified populations of neurons in vivo



The Goal:

Interstitial Cystitis / Bladder Pain Syndrome

IC/BPS:

Disabling

Pain on bladder filling

Urgency

Frequency



Instillation of Local Anesthetics



Short Duration of Action
Inconvenience
Risk of Infection

Sustained-Release Local Anesthetics



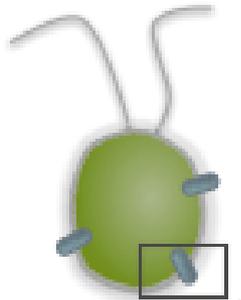
Nerve blocks for Treatment of Bladder Pain

- **The good**: Silencing neuronal activity works!
- **The bad**: we don't have a good way to do that chronically and safely
- **The Answer**:

Optogenetics
(of course)

How optogenetics works

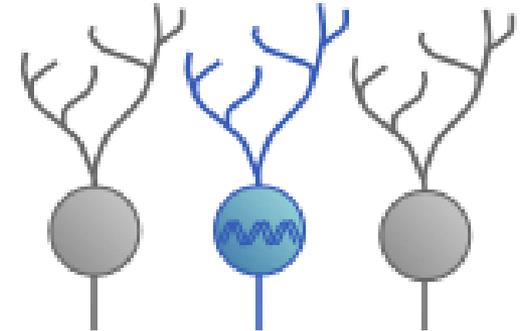
A light-sensitive protein from algae



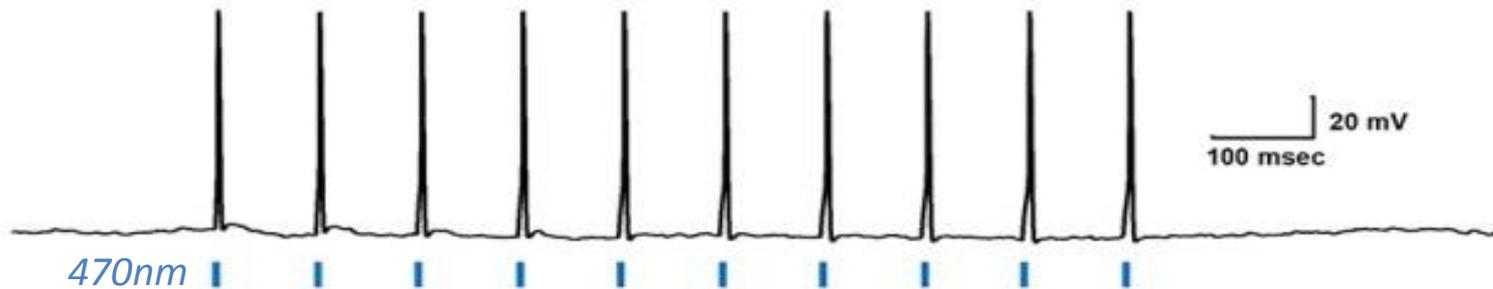
Take the gene for this protein...



... and insert the DNA into specific neurons in the brain

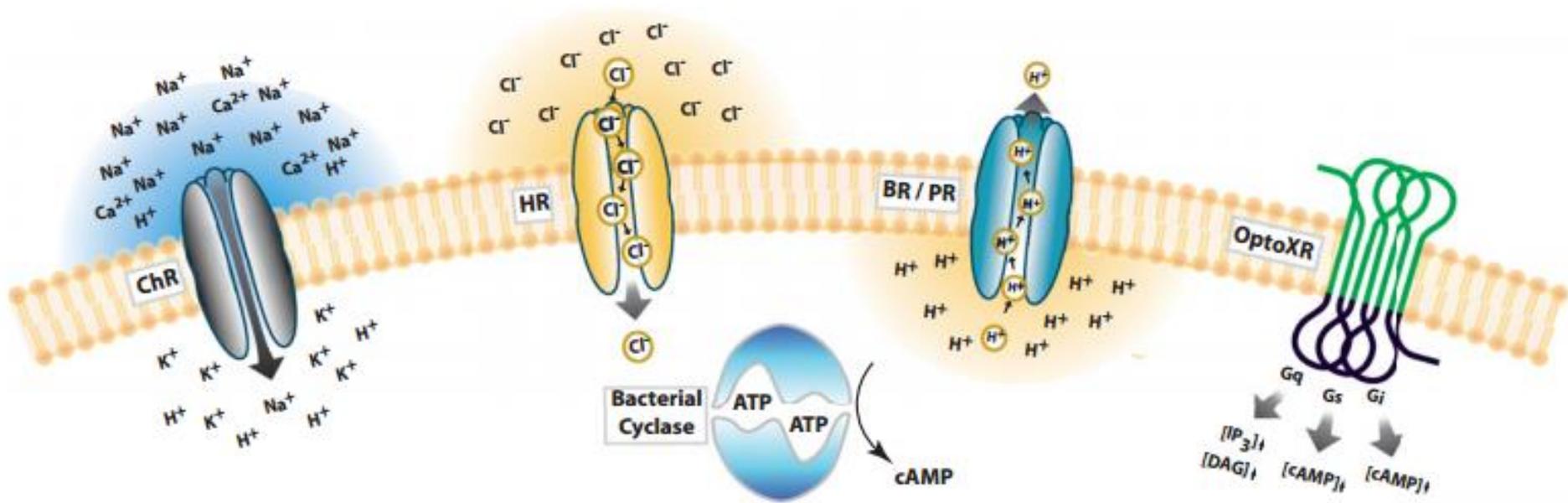


<http://neurobyn.blogspot.com/>



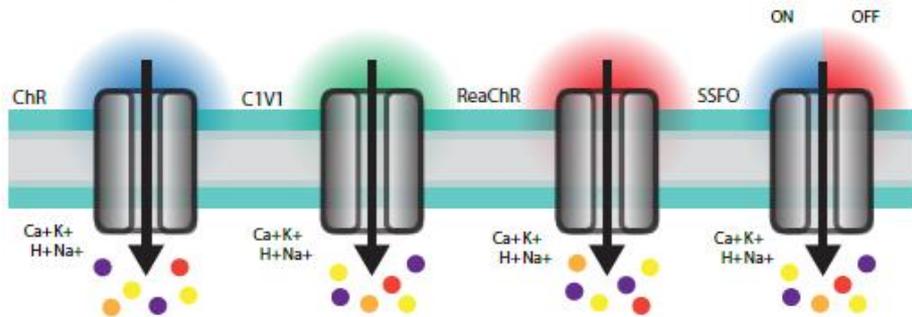
Bryan Copits

Optogenetics – Multiple “Opsins”

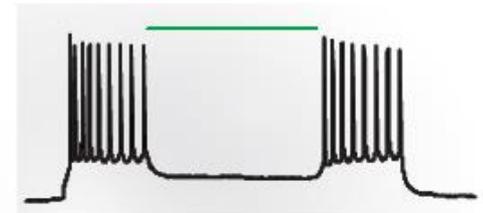
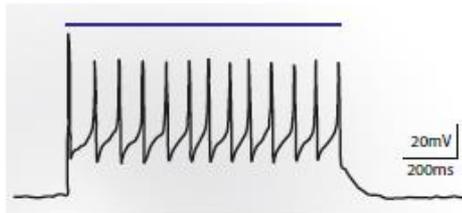
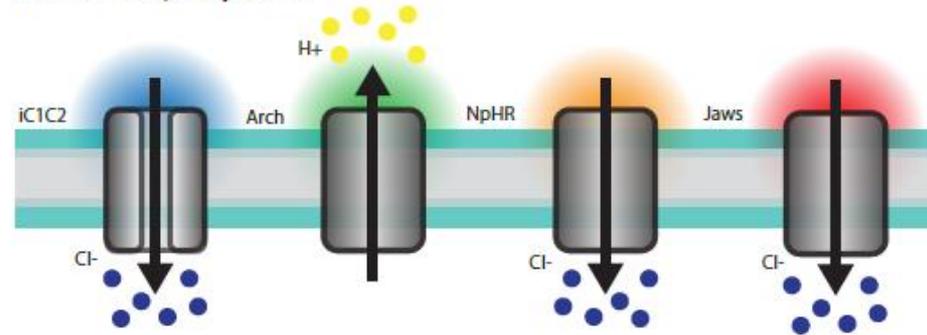


Optogenetics

Excitatory Opsins

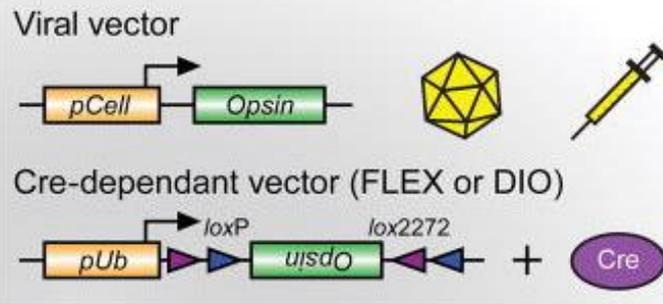


Inhibitory Opsins

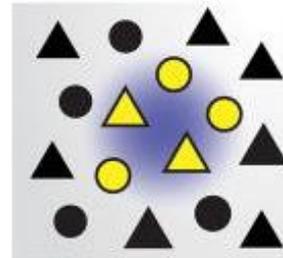


Optogenetics: Approaches to Expressing Opsins

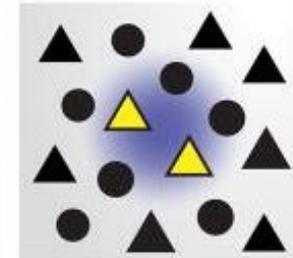
A Viral strategies



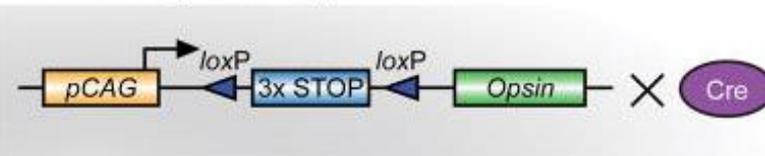
B Viral injection



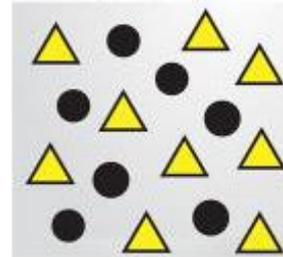
FLEX/DIO vector



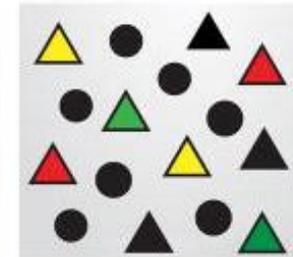
C Transgenic approach



D Transgenic line



INTRSECT

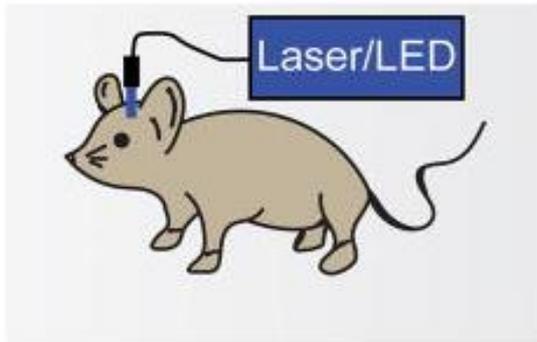


Optogenetics:

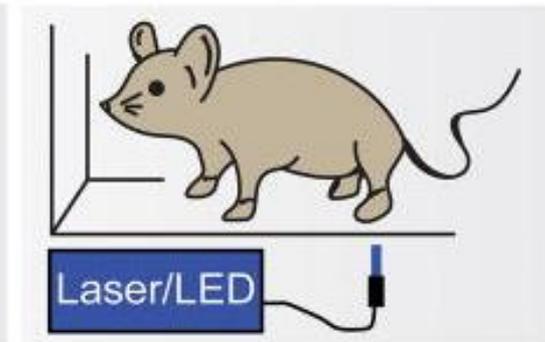
Approaches to Light Delivery

Traditional Light Delivery Methods

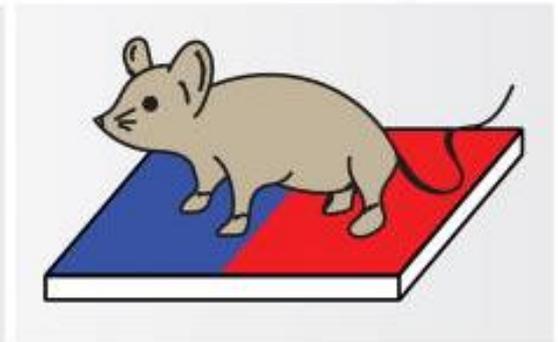
Implanted optical cable



External optical cable

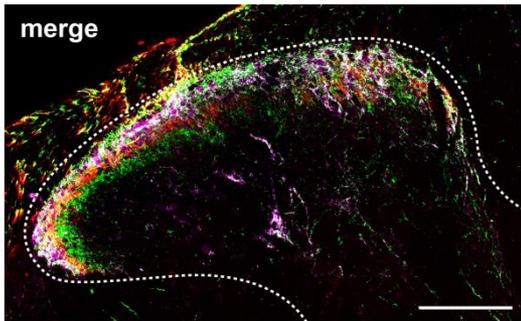


Floor LED array

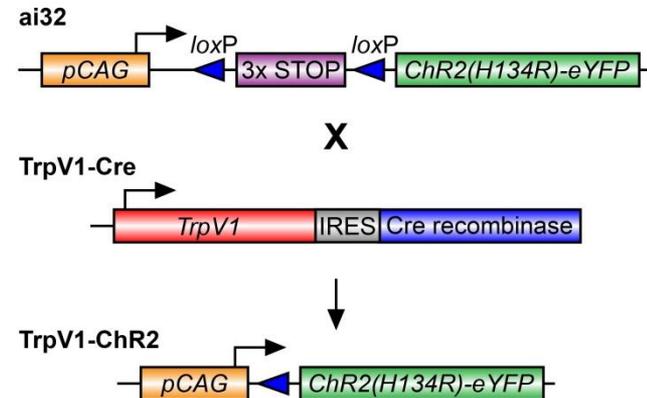
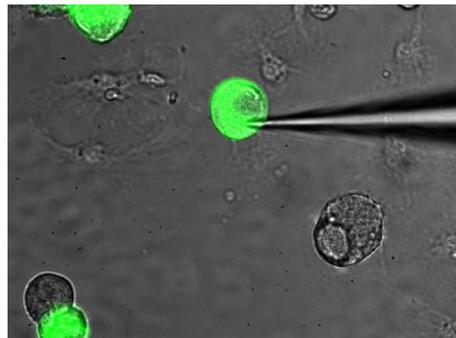
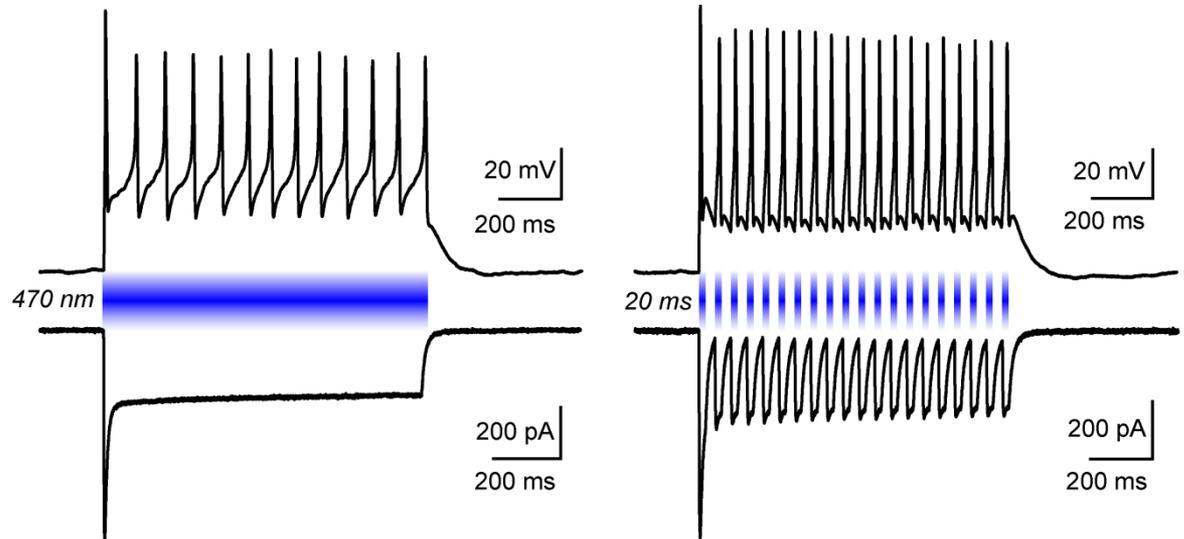
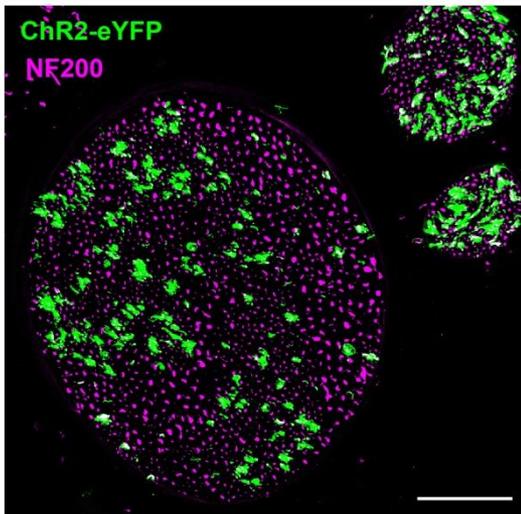
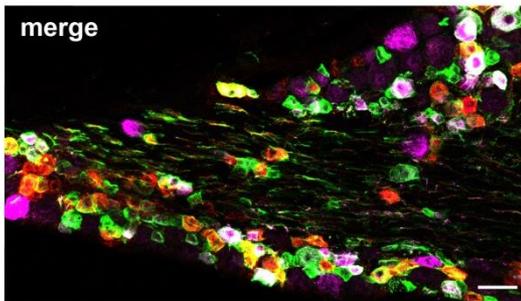


Can we use optogenetics to regulate pain circuits?

Light-Dependent Induction of DRG Firing by ChR2

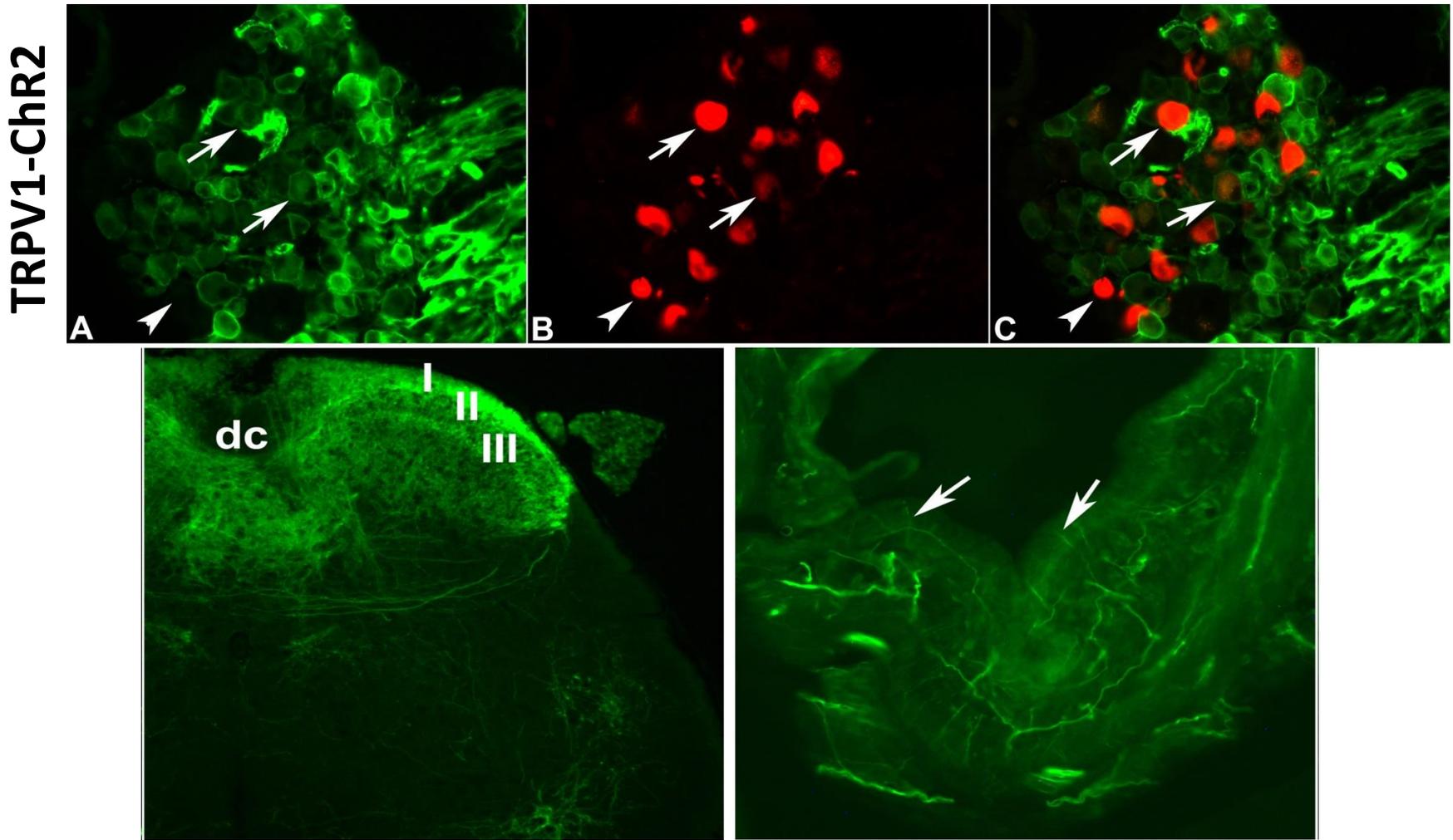


ChR2YFP / IB4 / CGRP



Similar to findings by Wang & Zylka, 2009

ChR2 expression from TRPV1 promoter: Expression in sensory neurons innervating bladder



L6 spinal cord

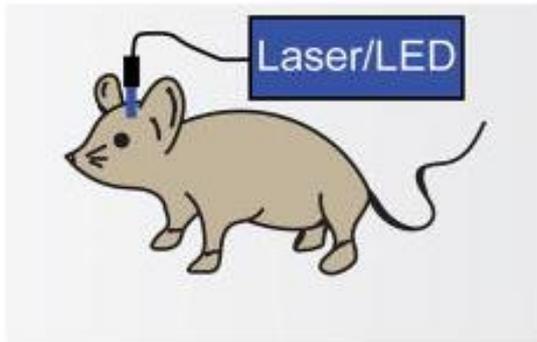
Bladder

Optogenetics:

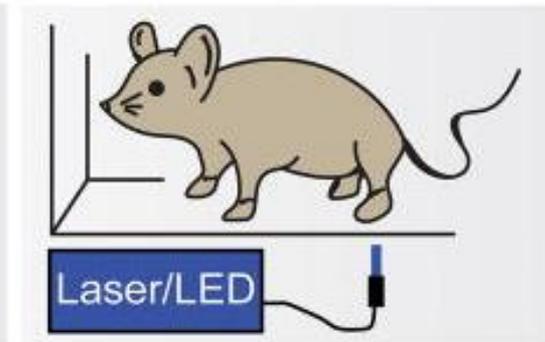
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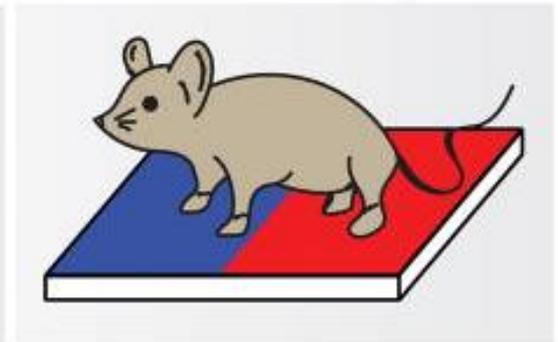
Implanted optical cable



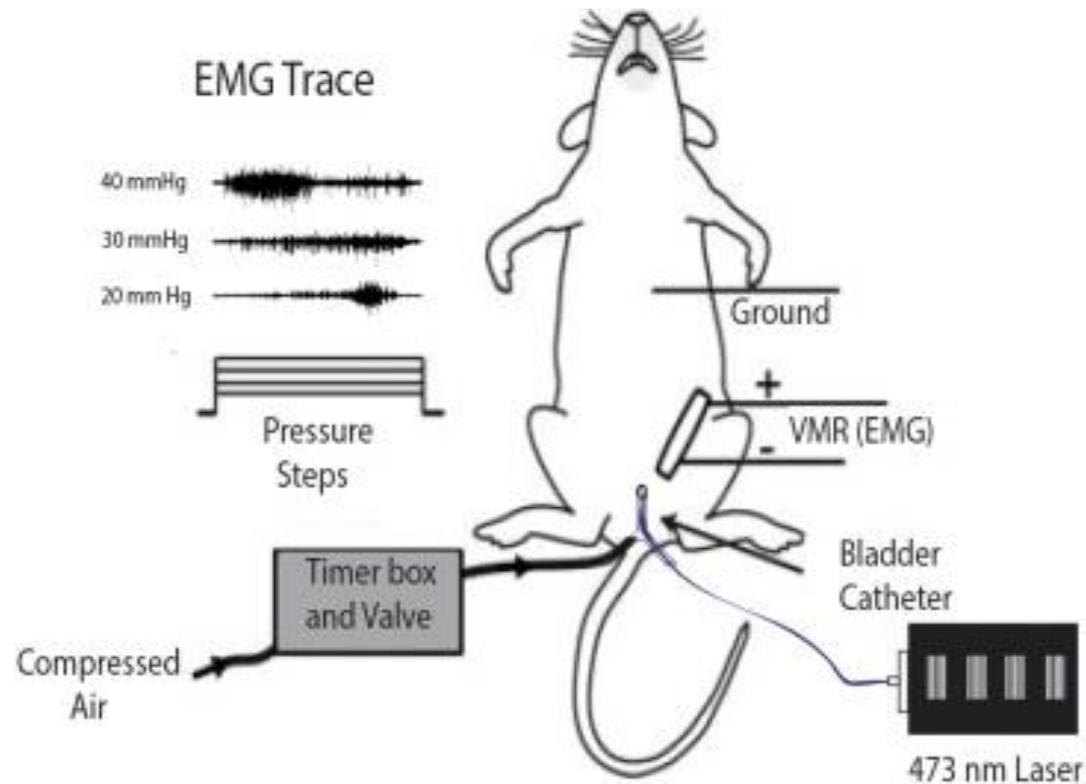
External optical cable



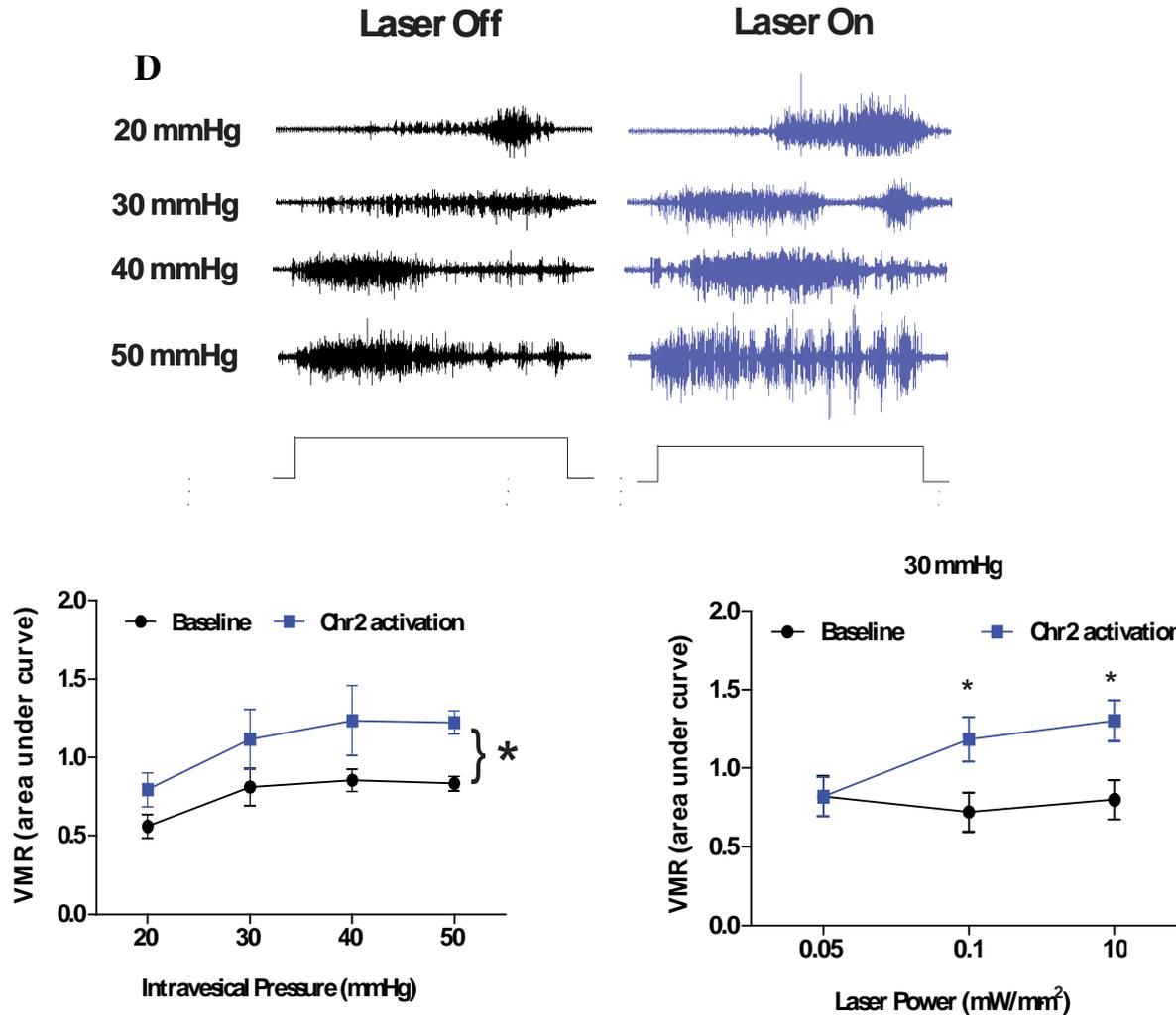
Floor LED array



Fiber optic Illumination of the Bladder for Optogenetic Modulation of Distension- Induced Bladder Pain (VMR)



ChR2 activation in TRPV1 bladder sensory neurons show significant increases bladder responses

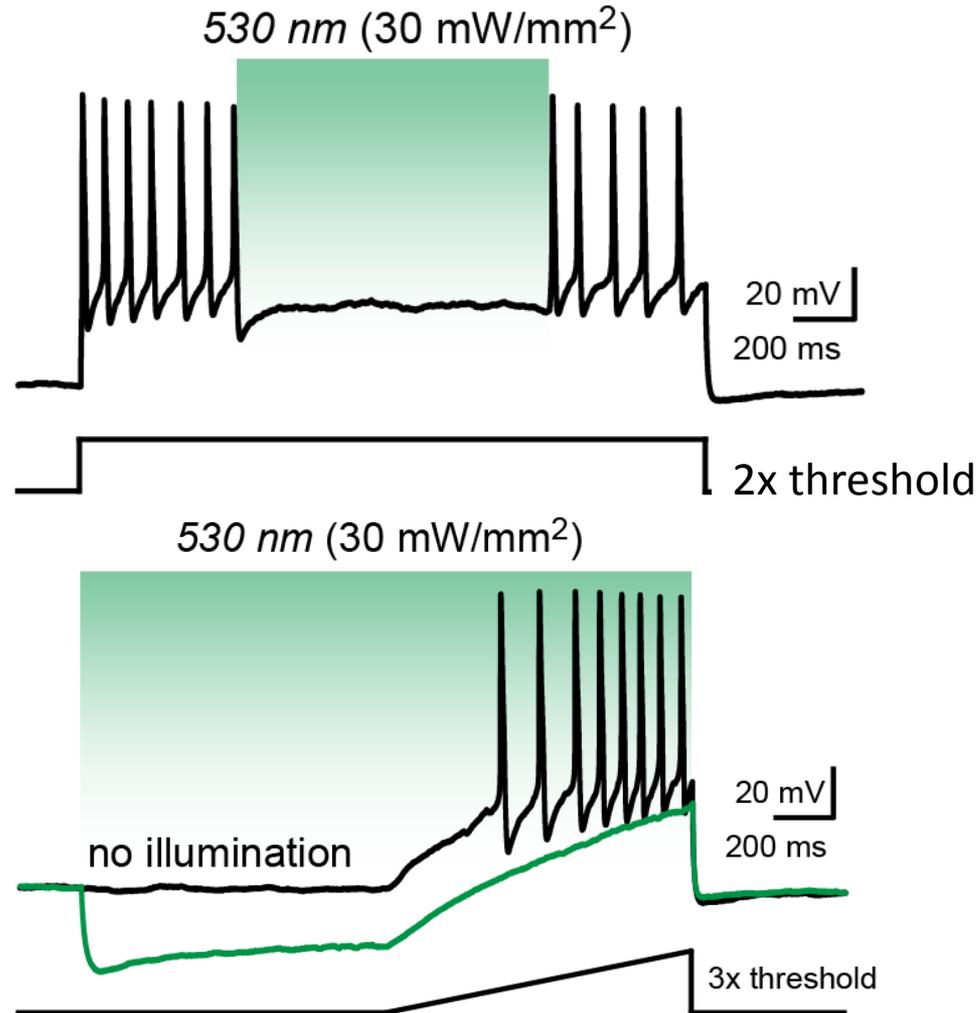


The Goal:

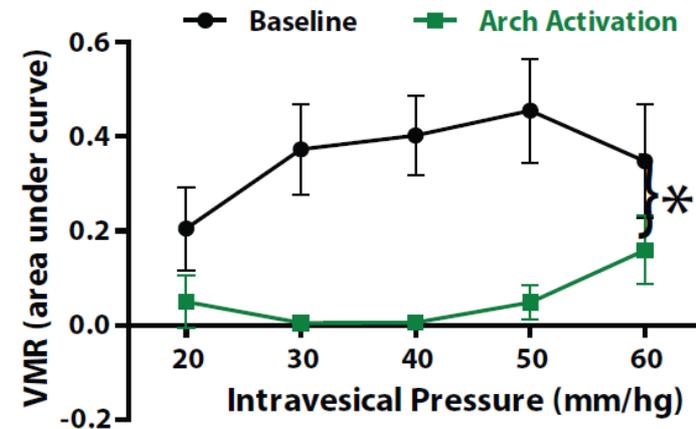
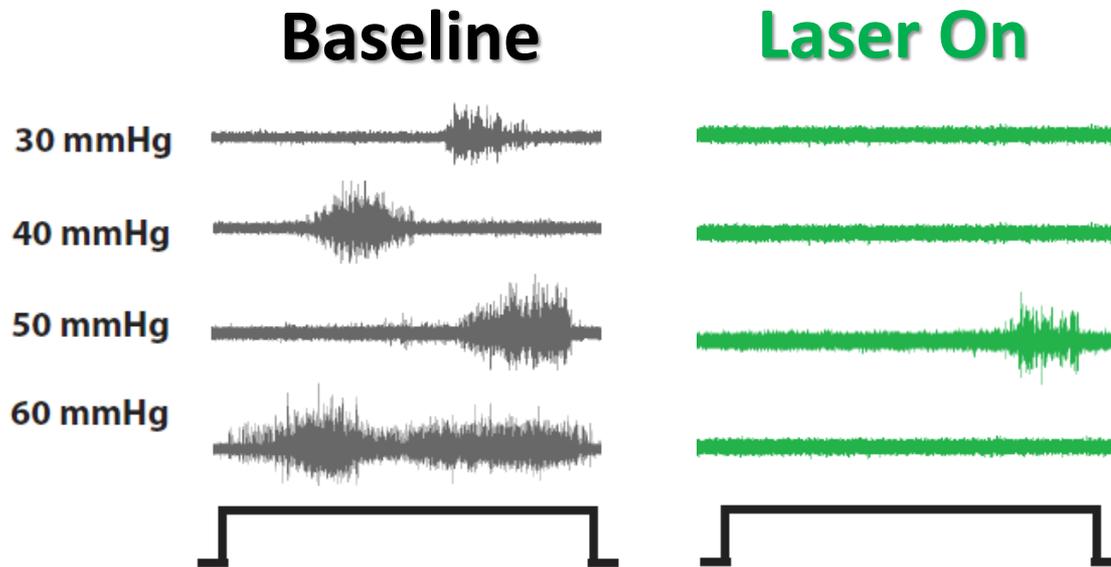
Interstitial Cystitis / Bladder Pain Syndrome



Light Activation of Archaelhodopsin Suppresses Firing in Bladder-Projecting Sensory Neurons



Light Activation of Archæorhodopsin Suppresses Firing in Bladder-Projecting Sensory Neurons



Declare Victory?

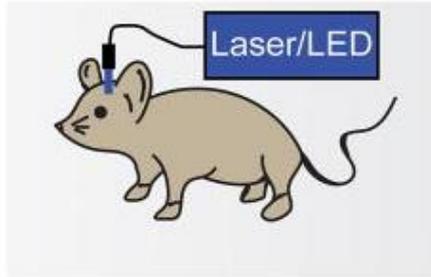
Fiber coupling leads to constraints on experimental design and significantly impacts animal behavior



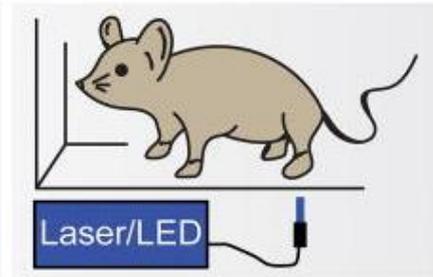
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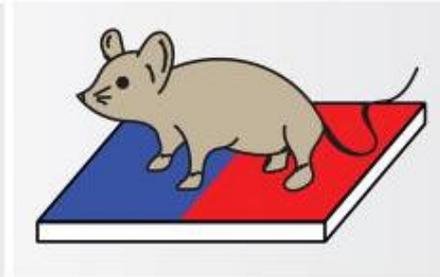
E Implanted optical cable



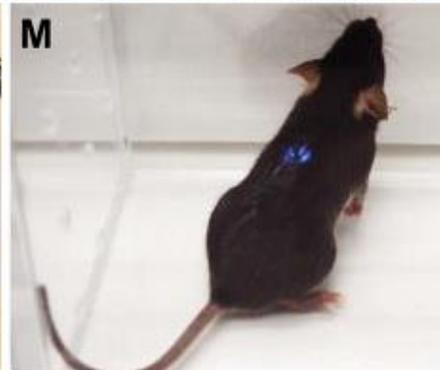
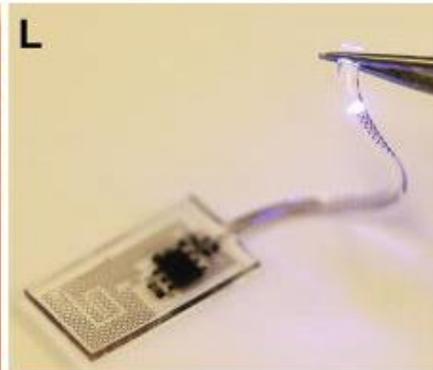
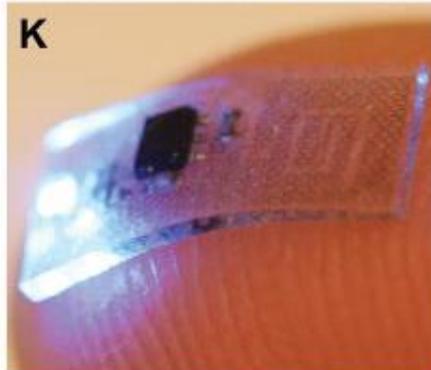
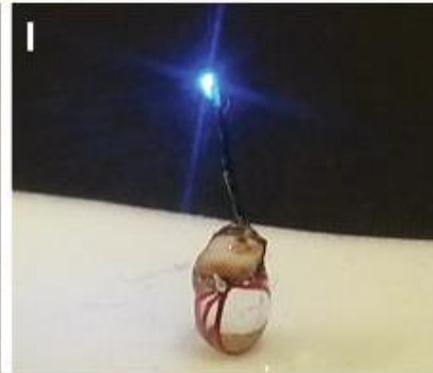
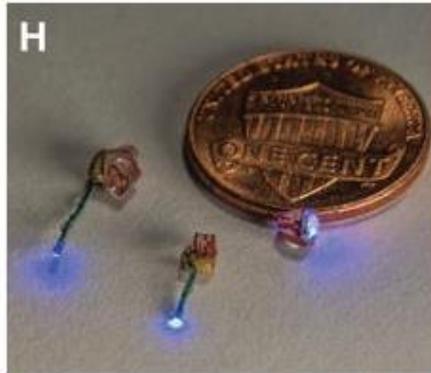
F External optical cable



G Floor LED array

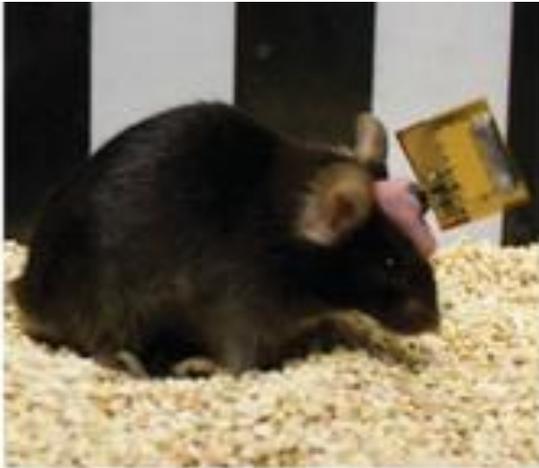
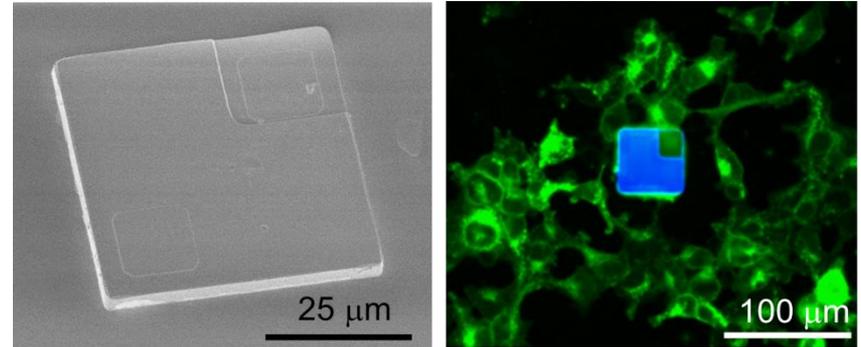


Wireless Implantable LED Devices



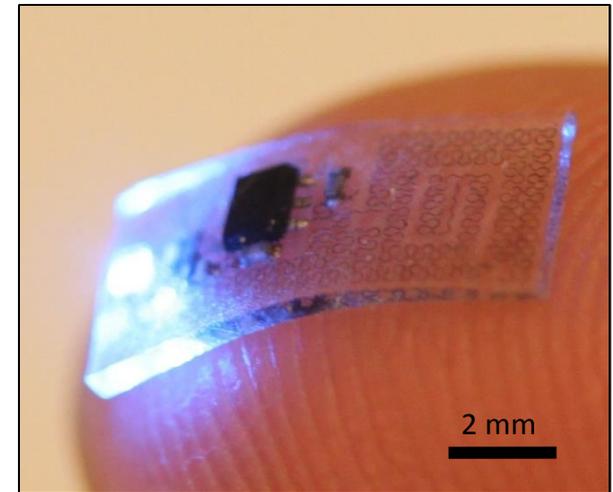
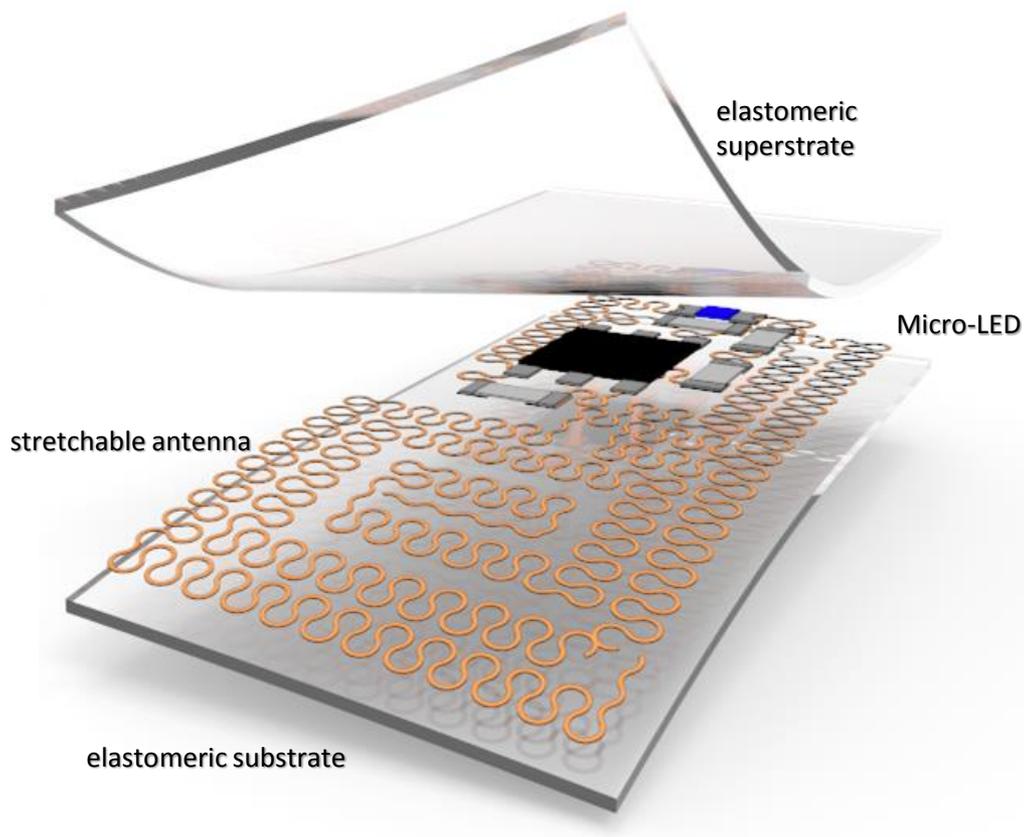
micro-LED Development

-collaboration with
John Rogers, U. Illinois

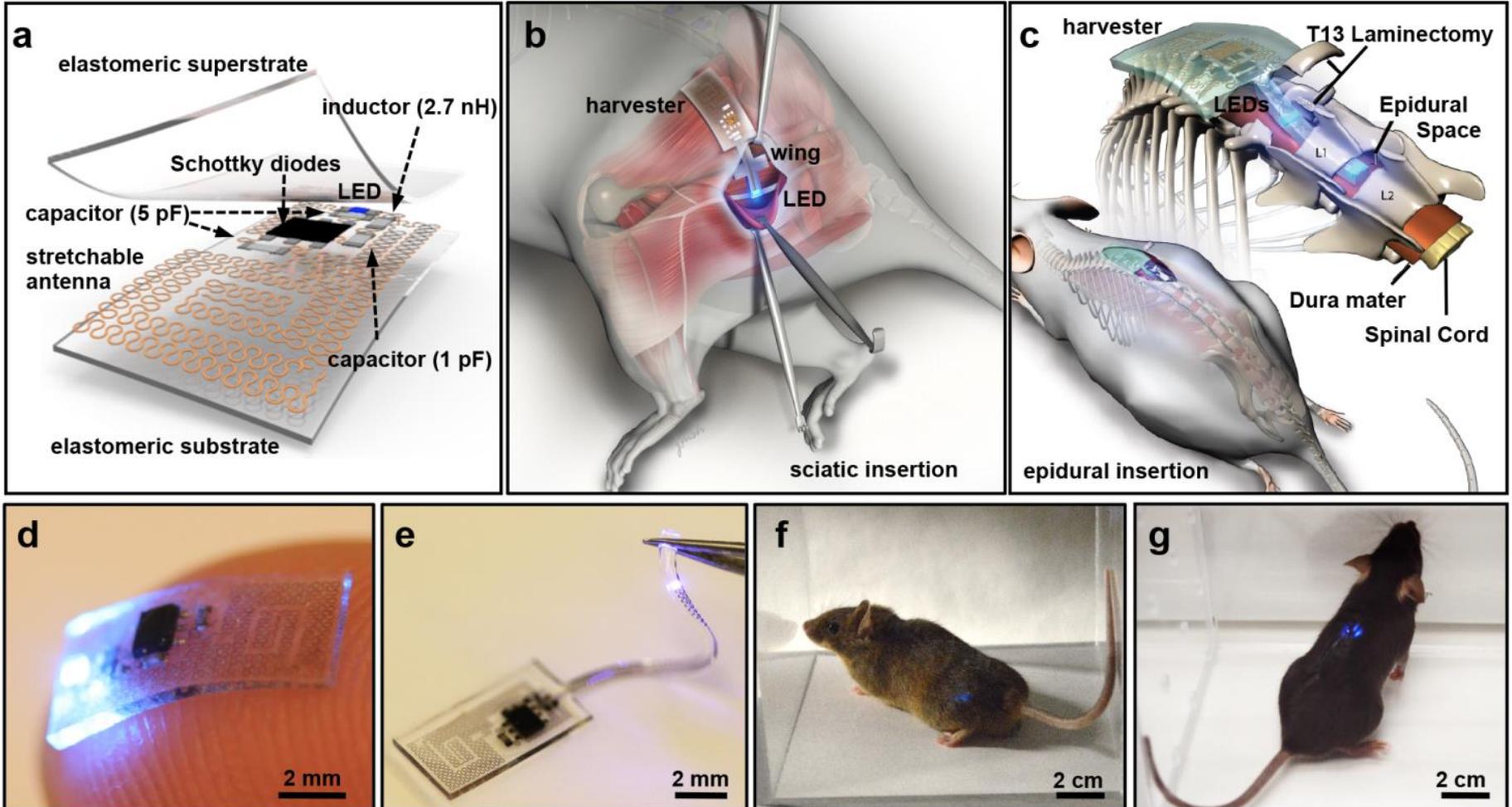


Kim et al, *Science*, 2013

Design of RF-Powered Fully Implantable Devices

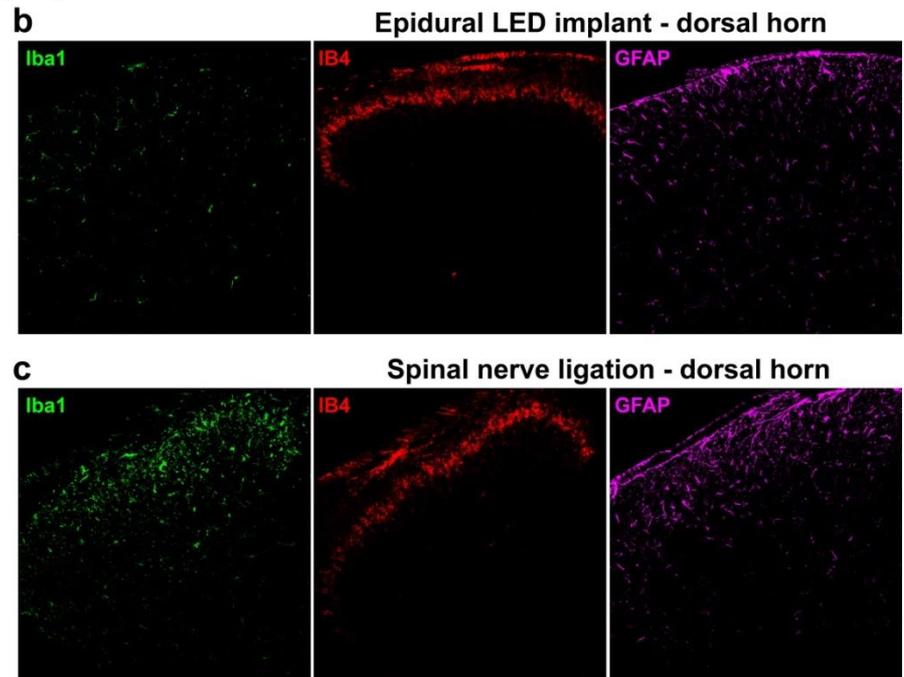
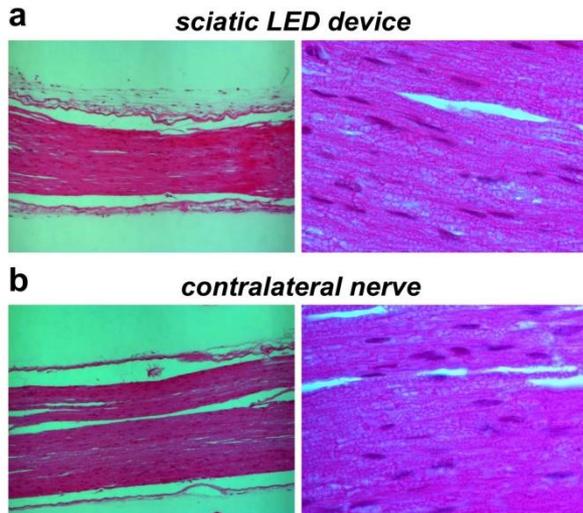


Soft Optoelectronics Systems for Diverse Wireless Optogenetic Applications

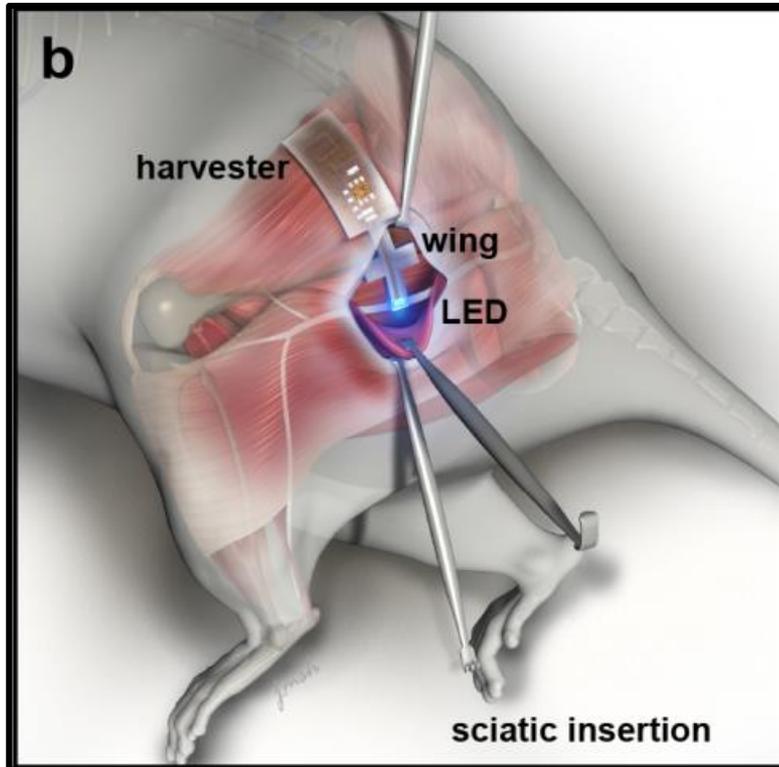


Wireless μ LED devices:

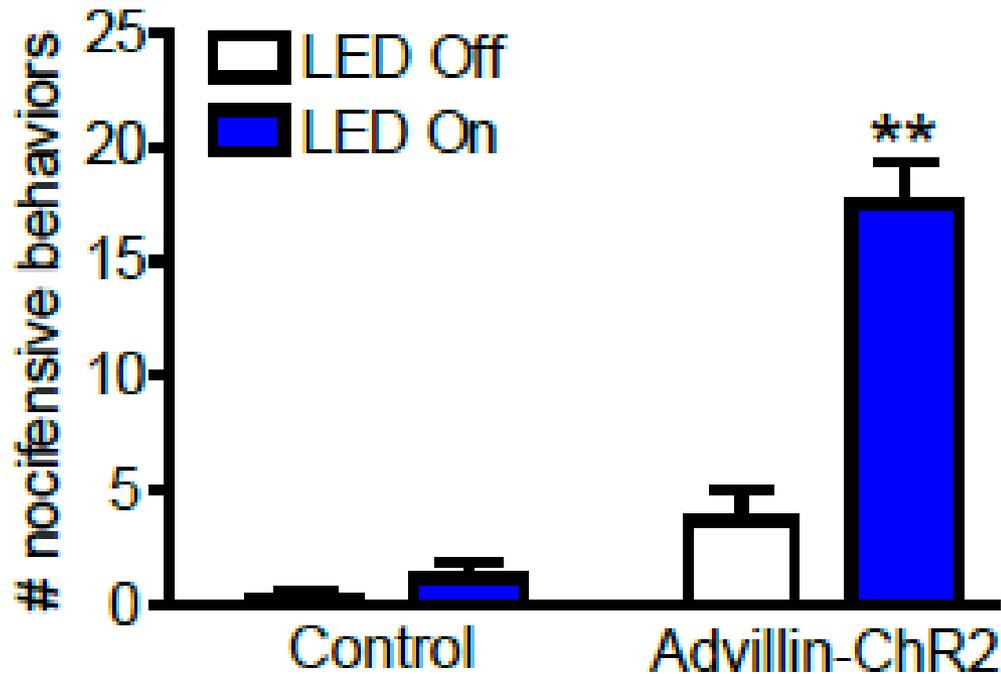
- No motor impairment
- Minimal heating ($<2^{\circ}\text{C}$)
- No inflammation or gliosis
- No nerve damage



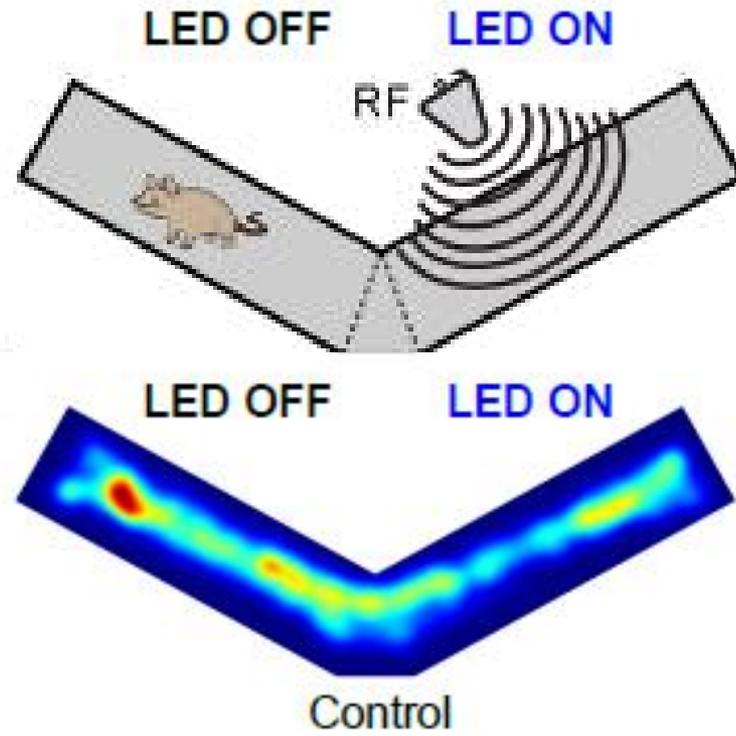
Fully Implantable RF-Powered Devices: Sciatic Application



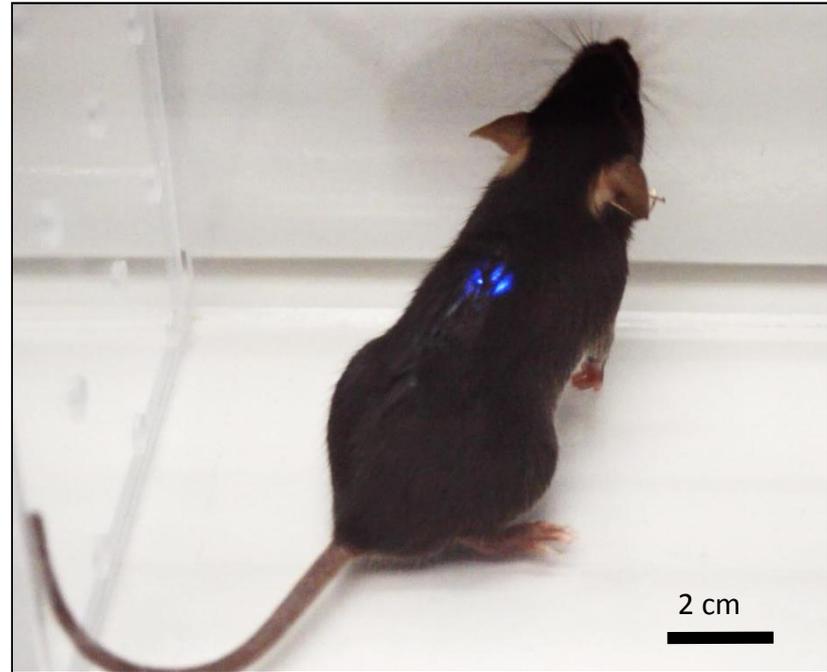
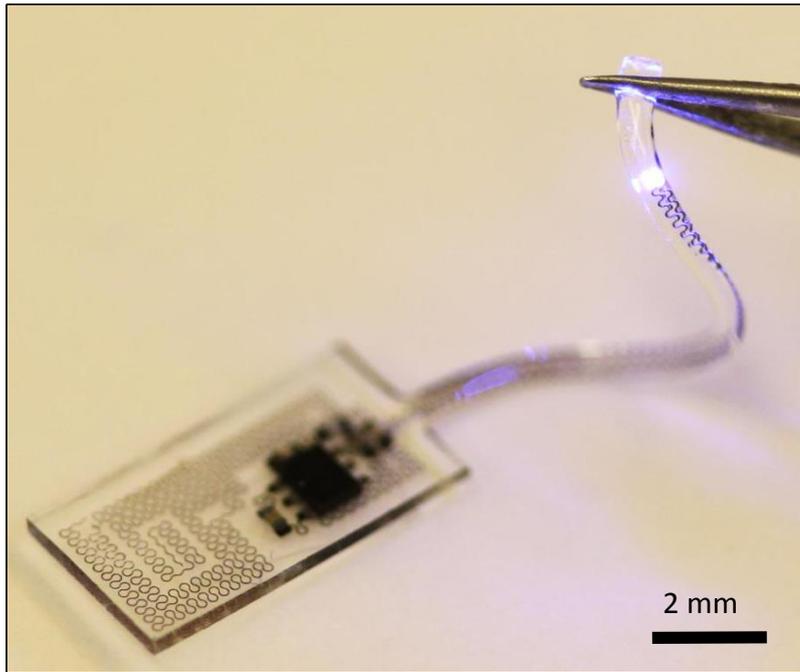
Activation of Device Implanted Over Sciatic Nerve Elicits Spontaneous Pain Behaviors



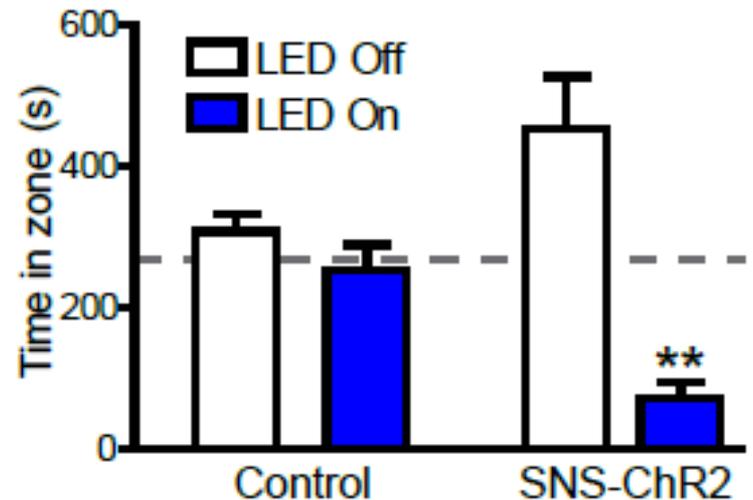
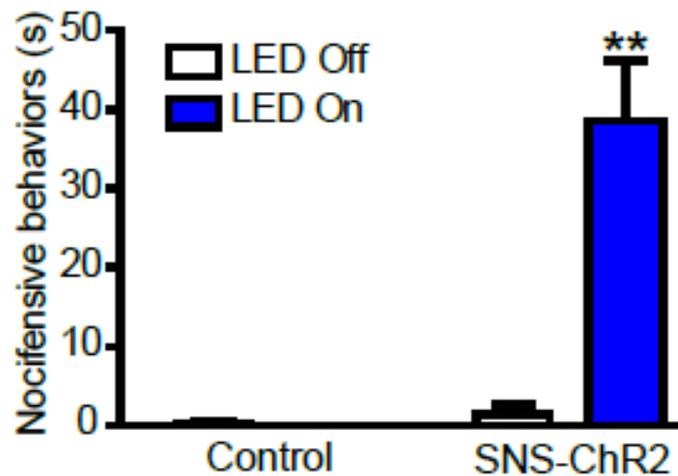
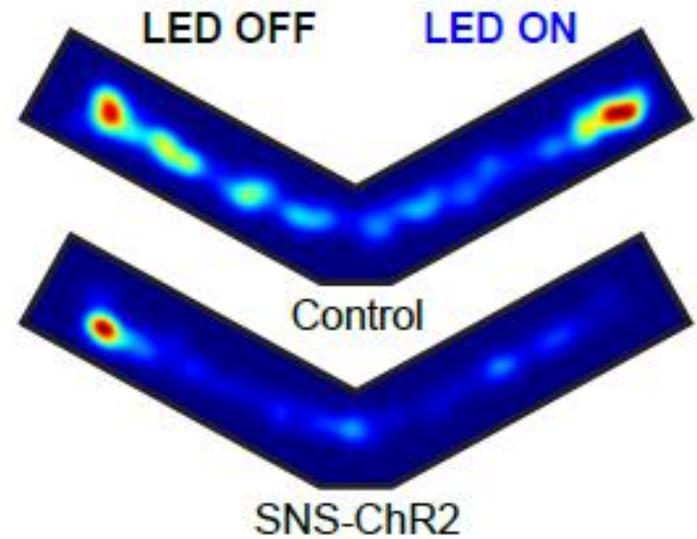
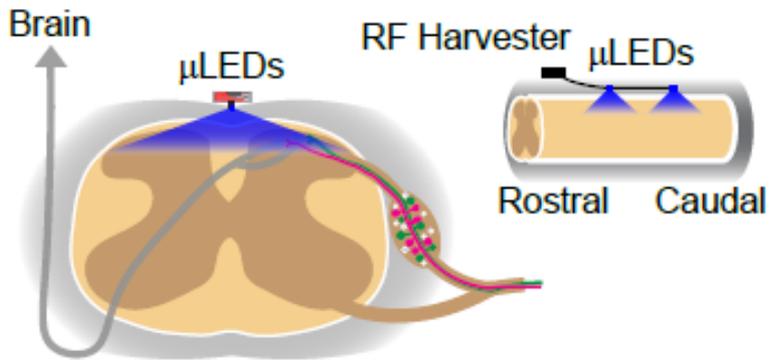
Activation of Sciatic Nerve Device Elicits Real-Time Place Aversion



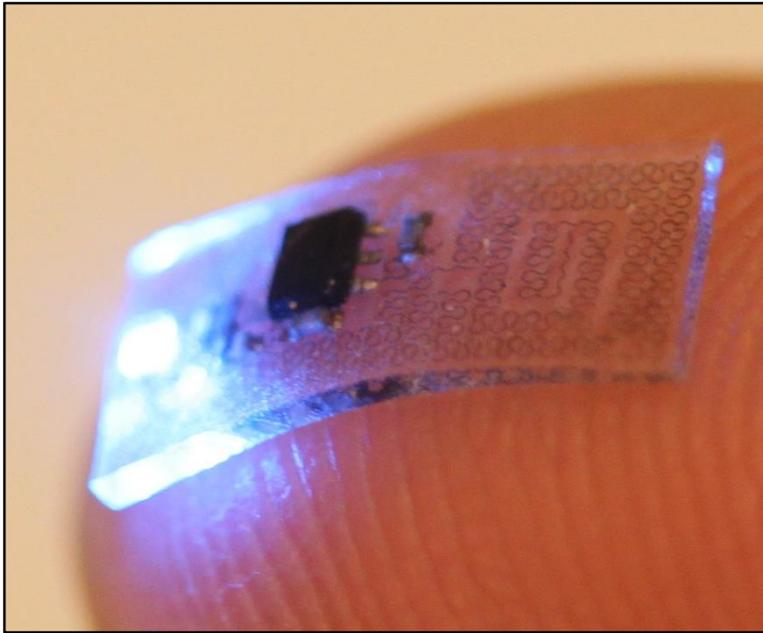
Fully Implantable RF-Powered Devices: Epidural Application



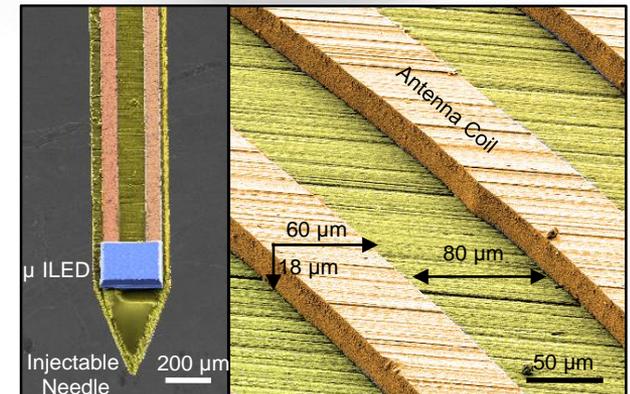
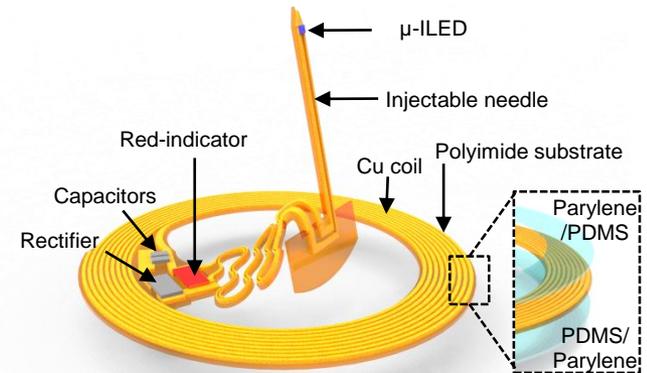
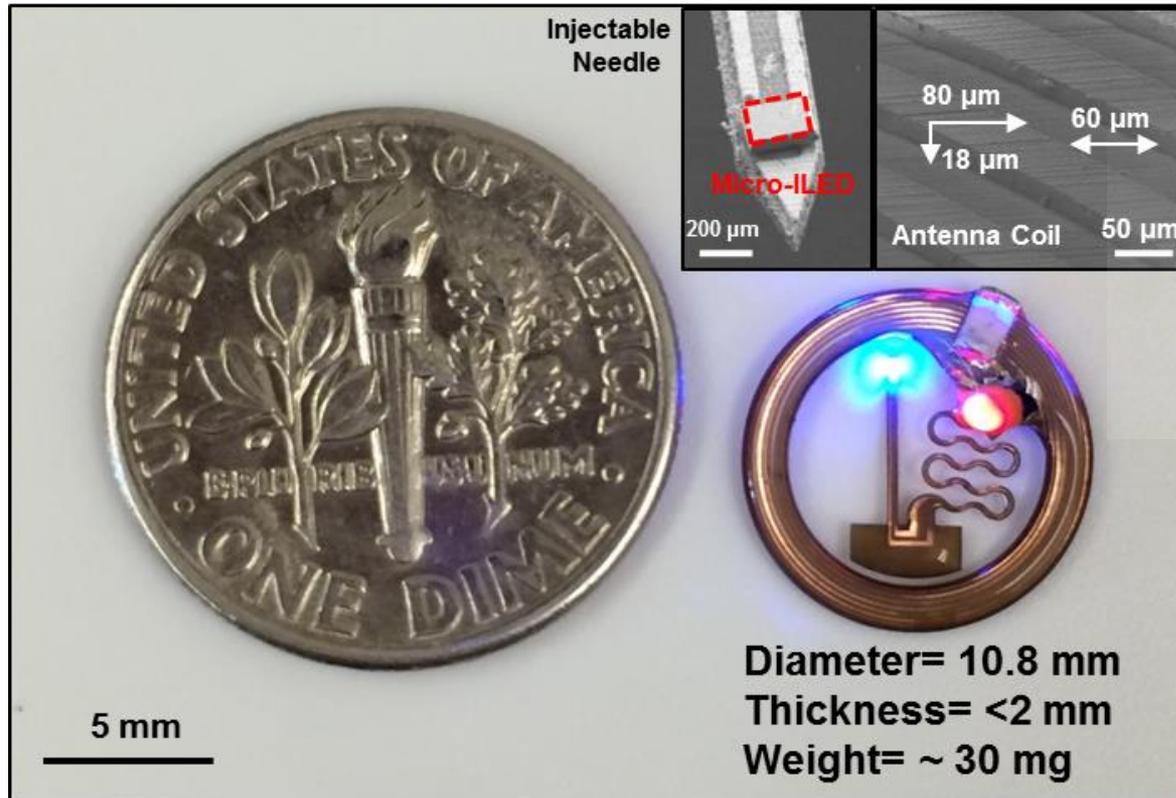
Activation of Epidural Device Elicits Spontaneous Pain and Real-Time Place Aversion



Fully Implantable RF-Powered Devices: Abdominal Application for Bladder Modulation

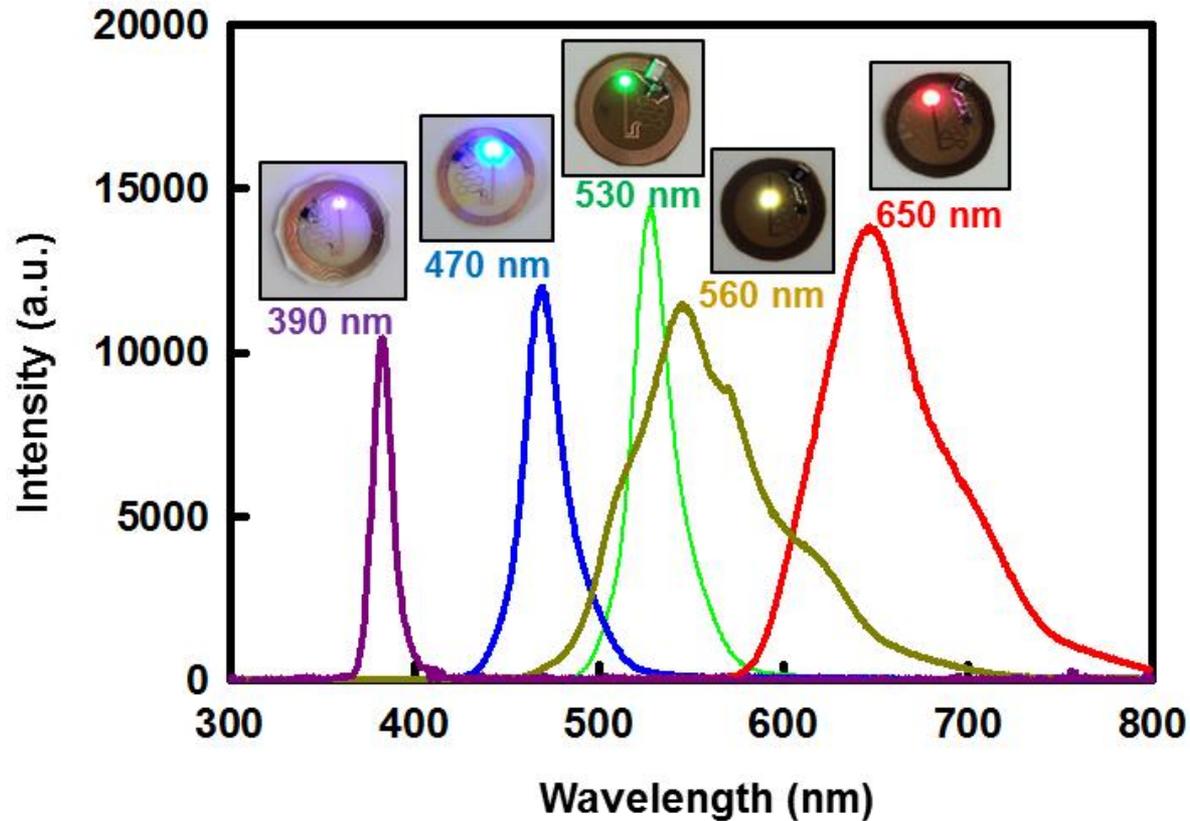


The Next Generation: Near Field Communication (NFC) Powered Devices



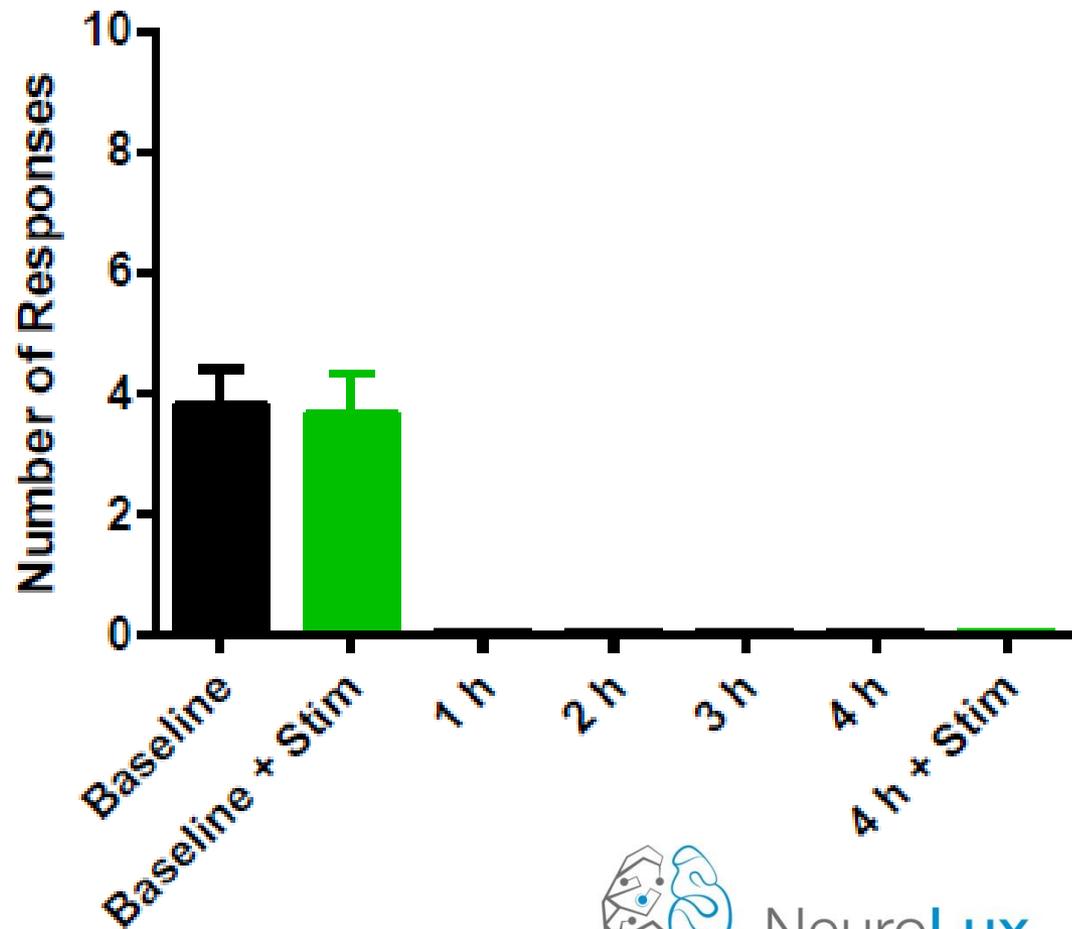
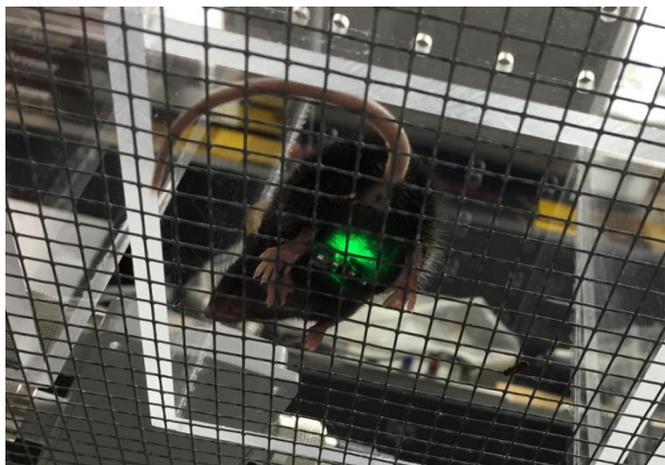
NeuroLux

NFC Powered Devices: Multiple Colors for Diverse Optogenetic Applications



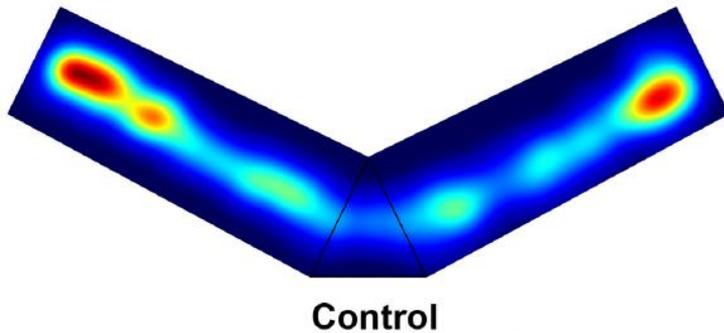
NeuroLux

micro-LED illumination of Bladder Afferents Expressing Arch under Nav1.8 Promoter: Effects on Cyclophosphamide (CYP)-Induced Referred Pain



Wireless Inhibition of Bladder Afferents in Mice with Bladder Pain induces *Real-Time* Place Preference

*Mice with CYP-induced cystitis, green NFC-microLED implants over bladder.
RTTP Allows Assessment of Relief of Ongoing Pain.*



LED Off

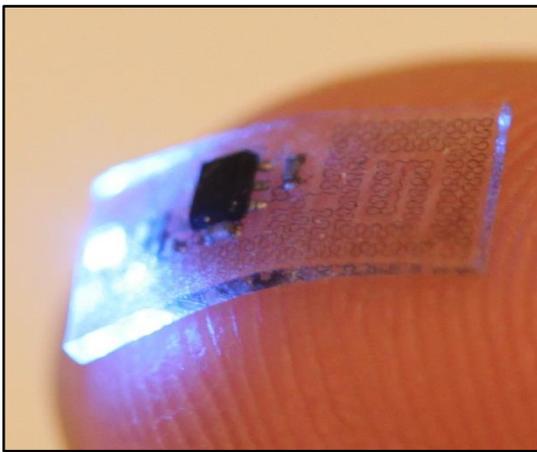


NeuroLux

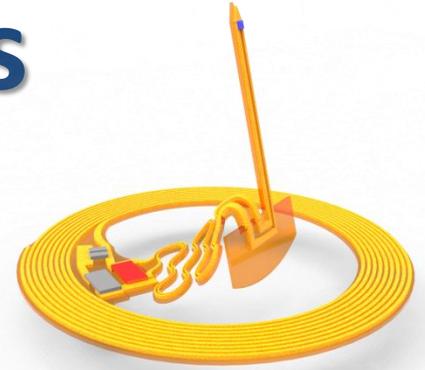
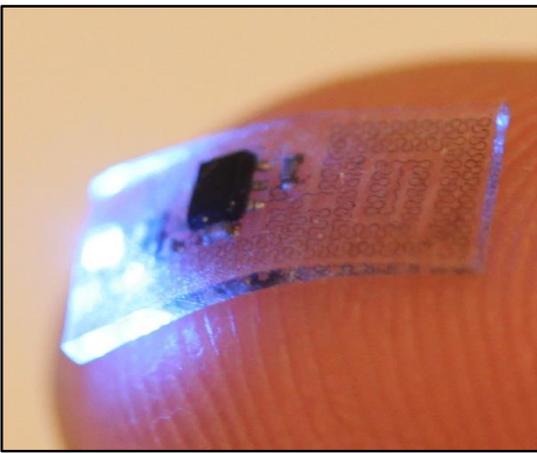
micro-LED illumination of Bladder Afferent Neurons in mice expressing ChR2 or Arch under various promoters:

TRPV1, Ret, CGRP, etc...

- Which afferent populations produce spontaneous pain, voiding behaviors?
- Which populations mediate sensitization to distension vs. “spontaneous” or ongoing pain?
- Can we perform fiber type-specific nerve blocks to manage pathological bladder pain?
- Can similar approaches be used to manage voiding dysfunction?



Conclusions

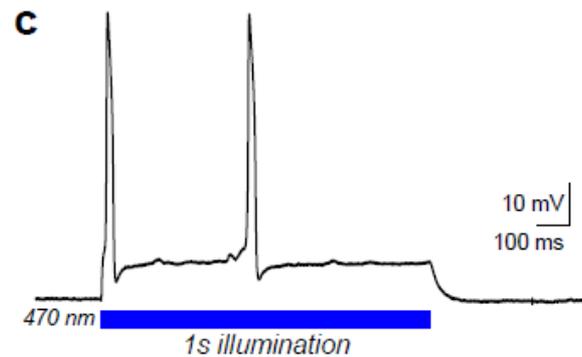
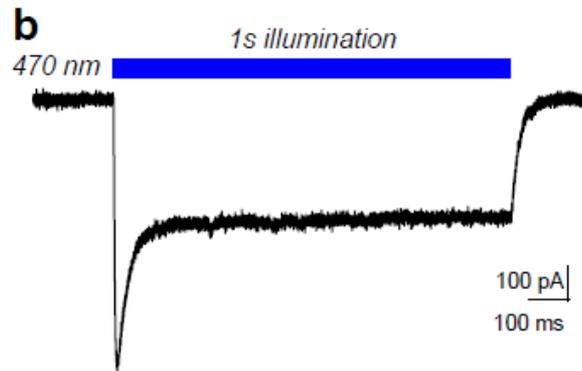


- Optogenetics offers a powerful approach to control activity of nociceptive afferent firing
- Fully-implantable RF-powered micro-LED devices offer a unique approach to study of pain
- **We demonstrate bidirectional modulation of bladder pain, including the demonstration of real-time place preference suggesting relief of ongoing pain**
- Viral gene therapy vectors to deliver opsins to bladder afferents, use in clinical management of bladder pain could be possible

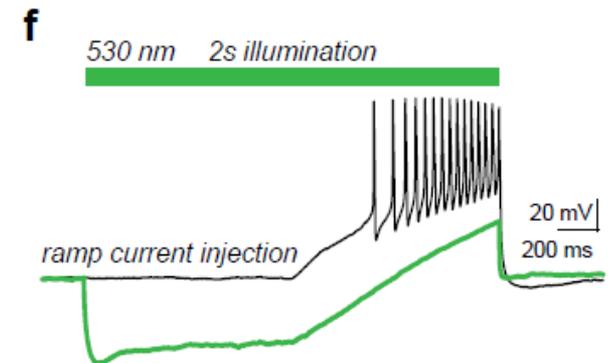
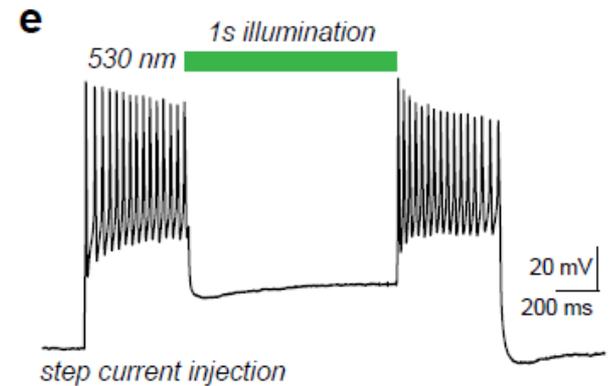
Optogenetic Modulation of Pain: Translation to Humans?

Human sensory neurons from organ donors, transduced with HSV vectors carrying ChR2 or halorhodopsin.

HSV::hChR2-eYFP



HSV::eNpHR3.0-eYFP





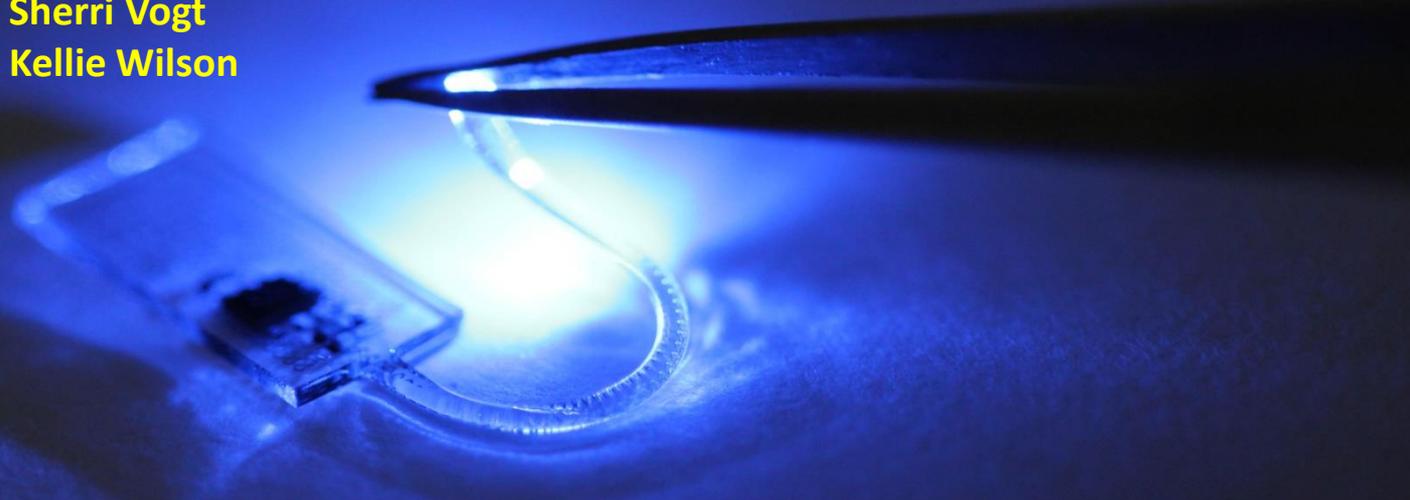
Gereau Lab

Dani Brenner, PhD
Bryan Copits, PhD
Steve Davidson, PhD
Judy Golden, PhD
Jose Grajales
Jordan McCall, PhD
Lisa McIlvried, PhD
Kajanna McKenzie
Kate Meacham, MD PhD

Aaron Mickle, PhD
Clint Morgan
Melanie Pullen
Vijay Samineni, PhD
Taylor Sheahan
Mani Valtcheva
Sherri Vogt
Kellie Wilson

Collaborators:

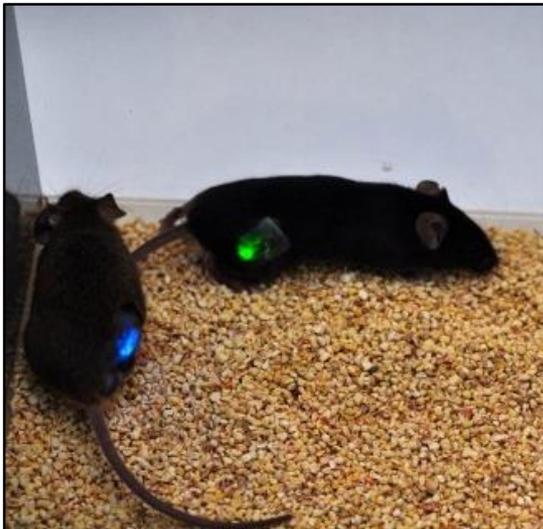
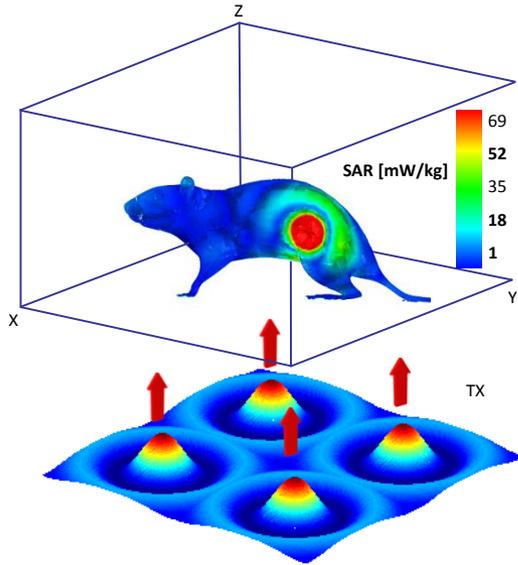
John Rogers Research Group (University of Illinois)
Michael R. Bruchas, PhD
Brian Davis (Pitt) and Jennifer DeBerry (UAB)
Henry Lai, MD & Gerald Andriole, MD



NIH National Institute of Neurological Disorders and Stroke

NIH National Institute of Diabetes and Digestive and Kidney Diseases

Stable optical output throughout a behavioral arena



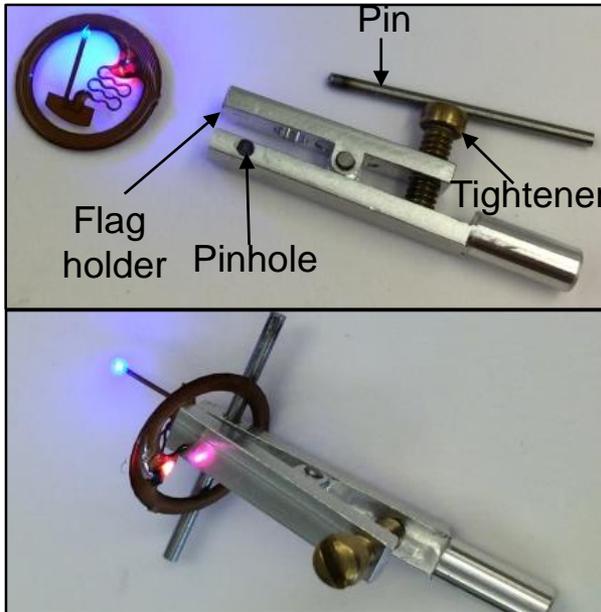
Near Field Communication (NFC) Powered Devices

Red-LED indicator

Micro-LED

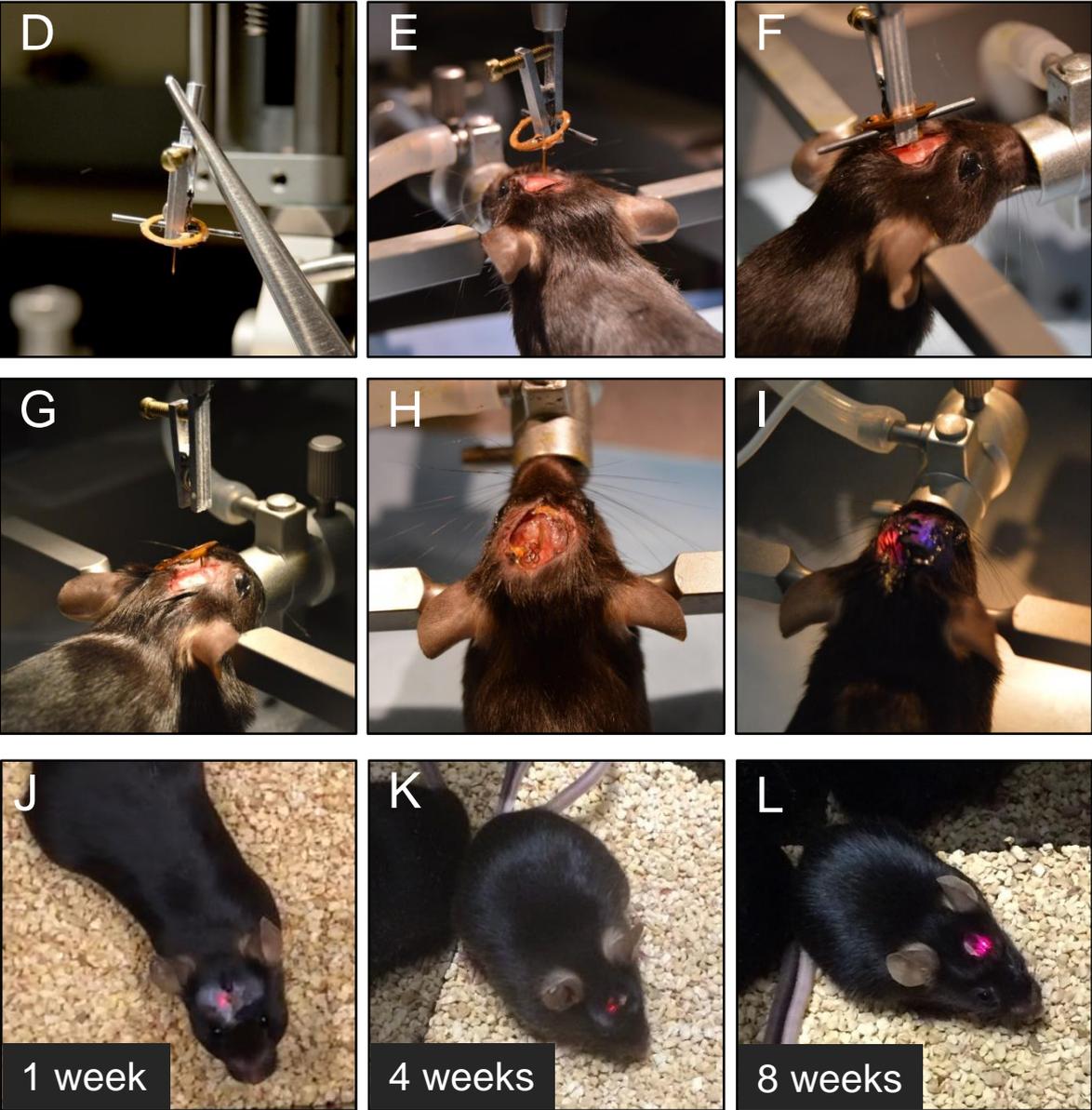
Injectable
Needle

Flag for
holding device

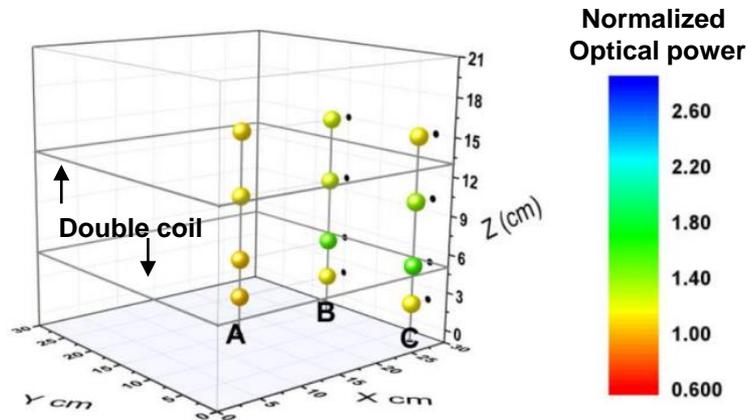


NeuroLux

NFC Devices: simple implantation, fast recovery

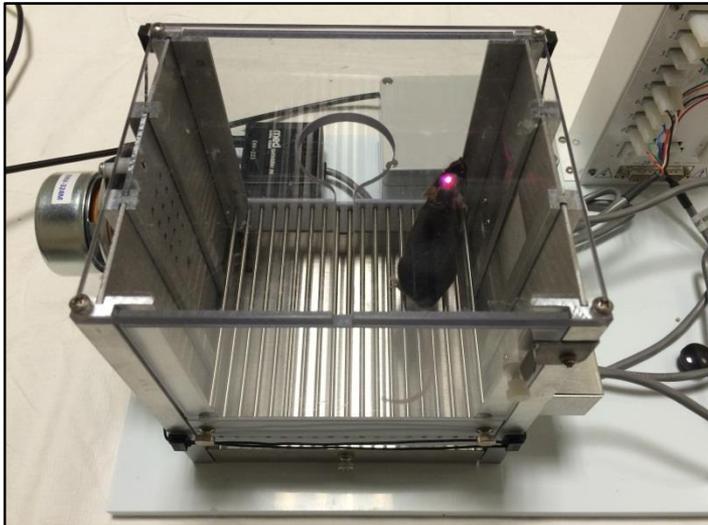


NFC Devices: versatile application



Coil position: 5 , 13 cm in z axis

Operant Chamber



Conditioned Place Preference Box



Real Time Place Preference Box



Homecage



Negligible Temperature Changes on Activation of Devices

